

Up-regulating the Human Intestinal Microbiome Using Whole Plant Foods, Polyphenols, and/or Fiber

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ABSTRACT: Whole plant foods, including fruit, vegetables, and whole grain cereals, protect against chronic human diseases such as heart disease and cancer, with fiber and polyphenols thought to contribute significantly. These bioactive food components interact with the gut microbiota, with gut bacteria modifying polyphenol bioavailability and activity, and with fiber, constituting the main energy source for colonic fermentation. This paper discusses the consequences of increasing the consumption of whole plant foods on the gut microbiota and subsequent implications for human health. In humans, whole grain cereals can modify fecal bacterial profiles, increasing relative numbers of bifidobacteria and lactobacilli. Polyphenol-rich chocolate and certain fruits have also been shown to increase fecal bifidobacteria. The recent FLAVURS study provides novel information on the impact of high fruit and vegetable diets on the gut microbiota. Increasing whole plant food consumption appears to up-regulate beneficial commensal bacteria and may contribute toward the health effects of these foods.

KEYWORDS: human gut microbiota, whole plant foods, polyphenols, fiber

INTRODUCTION

Epidemiologically, fruits and vegetables protect against chronic diseases including cardiovascular disease, cancer, obesity, and diabetes. Fiber, composed of plant structural and storage polysaccharides, and plant secondary polyphenolic compounds are thought to contribute significantly to the underlying protective processes.^{1–3} Interestingly, both potentially interact with the gut microbiota: for many polyphenols microbial transformation modifies bioavailability and activity, and fiber is the major energy source for fermentation in the colon, the dominant metabolic activity of the gut microbiota.

Plant polyphenols are a class of chemically diverse secondary metabolites that possess many different biological activities both within the plant and in the animals which eat these plants.⁴ Mainly because of their presence in foods and medicinal plants, they have long been studied for their interactions with mammalian physiological processes that play a role in chronic human disease. As well as being antioxidants and possessing inherent free radical scavenging abilities, plant polyphenols have the potential to affect certain risk factors of cardiovascular disease such as plasma lipid oxidation state, endothelial function, and platelet aggregation; protect against cancer by reducing DNA damage, cell proliferation, and metastasis; modulate immune function; inhibit bacterial pathogens; and protect against neurological decline (see Figure 1). The majority of the evidence for such health effects stems from animal studies using either whole plant foods or plant polyphenol extracts. Consequently, both the active chemical moiety and, in many cases, the underlying mechanism of action in humans remain to be determined.⁴ In this review, we will focus on interactions between polyphenols and fibers in whole plant foods with the human intestinal microbiota.

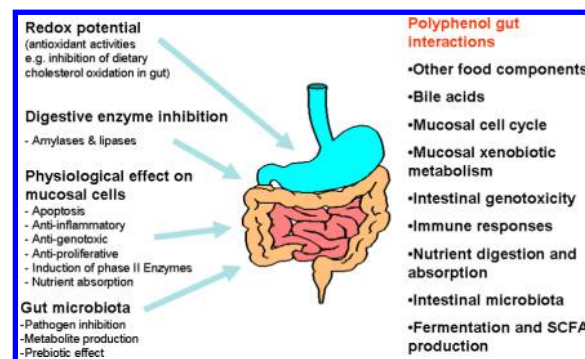


Figure 1. Schematic illustrating the direct intestinal effects of plant food polyphenols.

HUMAN GUT MICROBIOTA

The human gut microbiota is a diverse collection of microorganisms making up some 1000 species, with each individual presenting with their own unique collection of species. They play an important role in human health and disease, and interindividual variation in microbiota makeup influences the profile of metabolites released from dietary components that reach the colon and may also affect an individual's risk of chronic disease. Recent studies have identified a limited number of "enterotypes" within the human gut microbiota characterized by a predominance of *Prevotella*, *Bacteroides*, and/or *Ruminococcus*.^{5,6} Moreover, these

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enterotypes appear to be determined by long-term diet and could play an important role in an individual's risk of developing chronic diet-associated diseases such as obesity, metabolic syndrome, and certain cancers.⁶ Shotgun metagenomic sequencing has revealed differences in functional genes associated with these enterotypes, and these phenotypes appear to be linked to dietary fat, protein, fiber, and carbohydrate contents. Although no switching between enterotypes was observed during a small short-term dietary intervention with high-fat/low-carbohydrate or low-fat/high-carbohydrate diets, changes within the composition of the gut microbiota of the 10 subjects studied were observed after 24 h of dietary intervention. This indicates that even short-term dietary change can have a detectable impact on the makeup of the gut microbiome, in terms of both relative bacterial populations and the overall metabolic potential of the gut microbiota.⁶ Different profiles of gut bacteria have also been characterized in populations with chronic immune or metabolic-related diseases including inflammatory bowel disease (IBD), celiac disease, diabetes, and obesity.^{7,8} Typically, these conditions present with lower prevalence of beneficial butyrate-producing bacteria, such as *Faecalibacterium prausnitzii*, and the bifidobacteria, which appear to be indicative of a well-functioning, healthy saccharolytic type microbiota. These diseases are also often associated with high prevalence of Enterobacteriaceae, a phylum that includes many important gastrointestinal pathogens including *Escherichia coli*, *Shigella*, *Salmonella*, *Campylobacter*, and *Helicobacter*. These diseases too are often associated with increased intestinal permeability or "leaky gut", a process that appears to be regulated both by diet and by gut microbiota-associated characteristics.

Works in germ-free animals, animal models of obesity and the metabolic syndrome, and a limited number of human studies have shown that the gut microbiota of obese individuals differs from that of lean individuals (reviewed in refs 9 and 10). The obese appear to be typified by a gut microbiota with a reduced *Bacteroidetes*/*Firmicutes* ratio and perturbations within important fiber-degrading saccharolytic populations.¹¹ Indeed, this obese type microbiota has been shown to revert to a lean type profile with increased relative abundance of the *Bacteroidetes* when obese individuals lose weight on low-energy diets. Such microbiota compositional differences within the obese, and other disease states, are likely to have a dramatic impact on the colonic metabolic output and subsequent physiological processes in the host, including control of food intake, inflammation, energy storage, and energy expenditure (see Figure 1).¹¹ De Filippo et al.¹² recently characterized the gut microbiota composition of children living in rural Burkina Faso and urban Florence, Italy. The rural African children, following a traditional diet rich in whole plant foods, had a microbiota composition strikingly different from that of their European counterparts. Their microbiota was dominated by *Bacteroidetes*, notably the *Prevotella* and novel fiber-degrading species such as *Xylanibacter*, whereas the Italian children had a much lower ratio of *Bacteroidetes* to *Firmicutes*. The Italian children also had higher relative abundance of enterobacteria, including *E. coli*, *Shigella*, and *Salmonella*. In terms of microbiota metabolic activity, the African children had about 3-fold the concentration of short-chain fatty acids (SCFA) in their feces compared to the Italian urban children on a typical Western-style diet. Thus, the picture that is emerging is that although each of us carries our own unique collection of microorganisms, certain shifts or profiles of gut bacteria are

associated with poor diet, diets low in whole plant foods such as fruits and vegetables. Similar profiles of gut bacteria are also observed in chronic diseases of affluent Western lifestyles, such as obesity, metabolic syndrome, certain cancers, and autoimmune diseases such as IBD. We have recently shown that the type and quantity of dietary carbohydrate and fat can have a significant impact on the human gut microbiota and its metabolic output in people with metabolic syndrome.¹³ This highlights the real possibility of modulating the risk of metabolic disease by affecting interactions between diet and the gut microbiota.^{7,8,13}

Both the type and quantity of foods that escape digestion in the upper gut have an important impact on the composition and, therefore, activity of the colonic microbiota. Dietary fiber is the main source of carbohydrate for the gut microbiota. Fiber, usually as a result of its chemical structure or inaccessibility in whole plant foods, is naturally resistant to digestion in the upper gut. However, recent studies have shown that food-processing methodologies, including grain particle size upon milling or cooking processes, can modify both the digestibility of complex polysaccharides in foods and their impact on the human gut microbiota.^{14,15} Similarly, as much as 90% of plant polyphenols escape digestion and absorption in the upper gut and persist into the colon. Here they come into contact with the gut microbiota, acting as substrates for microbial production of small phenolic acids and SCFA or, indeed, affecting species composition and their metabolic activity.

■ FIBER AND THE GUT MICROBIOTA

In 2008 the European Commission presented a comprehensive definition of what constitutes dietary fiber. Commission Directive 2008/100/EC (28 October 2008) states that "fiber" means "carbohydrate polymers with three or more monomeric units, which are neither digested nor absorbed in the human small intestine and belong to the following categories: edible carbohydrate polymers naturally occurring in the food as consumed; edible carbohydrate polymers which have been obtained from food raw material by physical, enzymatic or chemical means and which have a beneficial physiological effect demonstrated by generally accepted scientific evidence; edible synthetic carbohydrate polymers which have a beneficial physiological effect demonstrated by generally accepted scientific evidence".

Importantly, this definition recognizes the role of dietary fiber in maintaining human health. Fiber is consistently found to be inversely associated with chronic human diseases such as cancer and cardiovascular disease in large-scale human epidemiological studies. Similarly, studies in laboratory animals have provided mechanistic data linking high dietary fiber (typically 10% w/w diet) and protection from these same diseases. This is particularly true for the prebiotic dietary fibers. The most recent definition of a prebiotic defines a dietary prebiotic as "... a selectively fermented ingredient that results in specific changes, in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health".¹⁶ Dietary prebiotics have been repeatedly shown in both animals and humans to modulate the gut microbiota using state of the art culture-independent microbiological techniques, and they typically increase the relative abundance of bifidobacteria. There is also strong animal data linking prebiotics with protection from metabolic syndrome, obesity, type 2 diabetes, colon cancer, and IBD and fortifying the gut microbiota against invading gastrointestinal pathogens.^{8,17}

Table 1. Examples of Important Plant Polyphenols and Their Microbial Catabolites^a

plant polyphenol	microbial catabolite	possible health effects
(-)-epicatechin ³⁷⁻³⁹	4-hydroxyphenylacetic acid 3-(3-hydroxyphenyl)propionic acid 5-(3,4-dihydroxyphenyl)- γ -valeric acid (-)-5-(3',4'-dihydroxyphenyl)- γ -valerolactone	antimicrobial/antimycotic activity in vitro antimicrobial activity against Gram-negative enterobacteria via outer membrane destabilization
(-)-epigallocatechin ³⁹	4-hydroxyphenylacetic acid (-)-5-(3',4'-dihydroxyphenyl)- γ -valerolactone	antimicrobial/antimycotic activity in vitro
(-)-epigallocatechin-3-O-gallate ³⁷⁻⁴²	pyrocatechol pyrogallol 4-hydroxyphenylacetic acid (-)-5-(3',4'-dihydroxyphenyl)- γ -valerolactone	antibacterial activity (especially against Gram-negative enterobacteria), acetylcholinesterase inhibition greater than gallic acid parent; inhibition of <i>Vibrio</i> spp. quorum sensing antimicrobial/antimycotic activity in vitro
chlorogenic acid ²⁵	hydrocaffeic acid dihydroxyphenyl acetic acid hydroferulic acid	reduced intestinal mucosal inflammation and oxidative damage in animal models of IBD
proanthocyanidins ²⁴	3,4-dihydroxyphenylpropionic acid 3-hydroxyphenylpropionic acid 3,4-dihydroxyphenylacetic acid	reduced inflammatory response from LPS-stimulated blood lymphocytes
curcumin ²⁶	ferulaldehyde	reduced inflammatory response from LPS-stimulated blood lymphocytes
daidzein ⁴³⁻⁴⁶	equol	phytoestrogen important for heart and bone health and possible colon cancer protectants
daidzein ^{25,46}	O-demethylangolensin	estrogenic and/or antiestrogenic activity
quercetin ⁴⁶	2-(3,4-dihydroxyphenyl)acetic acid 2,3-(3-hydroxyphenyl)acetic acid 3,4-dihydroxybenzoic acid phloroglucinol 3-(3,4-dihydroxyphenyl)propionic acid 3-(3-hydroxyphenyl)propionic acid	
kaempferol ⁴⁶	2-(4-hydroxyphenyl)acetic acid	
naringenin ⁴⁶	3-(4-hydroxyphenyl)propionic acid phloroglucinol	antimicrobial activity against Gram-negative enterobacteria via outer membrane destabilization
isoxanthohumol ⁴⁶	8-prenylnaringenin	
catechin and epicatechin ^{37,46}	3-(3-hydroxyphenyl)propionic acid 5-(3',4'-dihydroxyphenyl)- γ -valerolactone 5-(3'-hydroxyphenyl)- γ -valerolactone 3-hydroxyhippuric acid pyrogallol 5-(3,4-dihydroxyphenyl)valeric acid 5-(3-hydroxyphenyl)valeric acid 3-(3,4-dihydroxyphenyl)propionic acid 5-(3-methoxyphenyl)valeric acid 3-(3,4-dihydroxyphenyl)propionic acid 5-(3-methoxyphenyl)valeric acid 2,3-dihydroxyphenoxy 3-(3',4'-dihydroxyphenyl)propionic acid	antimicrobial activity against Gram-negative enterobacteria via outer membrane destabilization
ellagitannins/ellagic acid ^{3,22,25,47}	uroolithin-A uroolithin-B uroolithin-C	estrogenic and/or antiestrogenic activity, antimalarials, induction of detoxification enzymes estrogenic and/or antiestrogenic activity, antimalarials, induction of detoxification enzymes estrogenic and/or antiestrogenic activity

Table 1. continued

plant polyphenol	microbial catabolite	possible health effects
	uroolithin-D	estrogenic and/or antiestrogenic activity
rutin ^{37,48,49}	3-hydroxyphenylacetic acid 3,4-dihydroxybenzoic acid 4-hydroxybenzoic acid 3-(3-hydroxyphenyl)propionic acid 3,4-dihydroxyphenylacetic acid	rutin and catabolites inhibit AGE formation in vitro; antimicrobial activity against Gram-negative enterobacteria via outer membrane destabilization
lignans ⁴⁵	enterolactone enterodiols	phytoestrogen important for heart and bone health; possible colon cancer protectants phytoestrogen important for heart and bone health; possible colon cancer protectants

^aThe putative health effects of selected catabolites are also presented.

However, few human studies have been conducted with levels of dietary fiber or prebiotic demonstrated in animals to protect against these chronic diseases.

The majority of plant complex polysaccharides fall within the EC definition of dietary fibers, and estimates of fiber intake for Western populations are in the region of 20 g per day compared to populations consuming traditional diets rich in fruits, vegetables, and grains and our ancestral hunter-gatherers, who consume or consumed between 70 and 120 g fiber per day.¹ As well as plant nonstarch structural polysaccharides, which by virtue of their chemical makeup resist the degradative activities of human digestive enzymes in the stomach and small intestine, plant storage carbohydrates such as starch can also reach the colon when rendered physically or chemically inaccessible to human digestive enzymes.¹⁸ Food processing and food preparation processes such as cooking and cooling can affect greatly the proportion of dietary starch becoming resistant and reaching the colon. Similarly, other food components can affect the quantity of starch reaching the colon. For example, polyphenol-rich beverages when consumed at meal times can affect directly starch digestion by inhibiting starch-degrading enzymes in the upper gut and thus blunting postprandial glucose peaks. Similarly, the Maillard reaction in cooked foods can increase the recalcitrance of food macromolecules to digestion, leading to increased survival of both carbohydrates and proteins until the colon.⁸ However, once these food compounds reach the colon, they become available to the fermentative activities of the human colonic microbiota. Recent metagenomic studies have highlighted that the gut microbiota is specifically evolved for the digestion of complex plant polysaccharides, possessing a range of polysaccharide- and glycan-degrading enzymes not present in the human genome.^{6,19} In this way, the human gut microbiota can be viewed as a closely coevolved microbial partner to the human genome, extending host-encoded functions and allowing the host to derive energy and other biologically active compounds from food components that would otherwise remain inaccessible and be excreted as waste.

Carbohydrate fermentation is the chief energy source for the gut microbiota, and the proximal colon can be viewed as a saccharolytic environment, where the dominant fermentative activity is carbohydrate fermentation leading to the production of the short-chain fatty acids acetate, propionate, and butyrate. These small organic acids have diverse functions in the host, not just supplying energy to the intestinal mucosa, heart, brain, and muscle but also playing important roles in human cell differentiation, proliferation, and programmed cell death; regulation of immune function; thermogenesis; and lipid

metabolism.¹⁰ In individuals following a Western style diet at least, as dietary fiber and colonic carbohydrate are used up in the proximal colon, saccharolytic fermentation decreases along the transverse and distal colon as the concentration of substrate decreases. Microorganisms then switch to other energy sources including dietary- or host-derived proteins and amino acids. The end products of amino acid fermentation include SCFA but also branched-chain fatty acids, amines, indoles, sulfides, and phenols, some of which are potentially harmful, being variably genotoxic, cytotoxic, and carcinogenic. As described above, human populations with higher intakes of dietary fiber and whole plant foods tend to have higher concentrations of SCFA in their feces, suggesting that in these populations carbohydrate fermentation may be extended along the length of the colon. This may thus avoid buildup of toxic or harmful metabolites produced when bacteria switch their fermentation substrate from carbohydrate to amino acids. We have recently shown that a 3-fold increase in dietary fiber results in a proportional increase in SCFA production by the gut microbiota and extends saccharolytic fermentation into the transverse and distal colon using an in vitro three-stage model of the human colonic microbiota.²⁰ It would be very interesting to confirm in humans following a low-fiber Western style diet whether intervention with high levels of dietary fiber can modulate the health-promoting saccharolytic activities of the gut microbiota to a similar degree in vivo.

■ POLYPHENOLS AND THE HUMAN GUT MICROBIOTA

Hydrolysis by the gut microbiota can increase the bioavailability and possibly, the biological activity of polyphenols reaching the colon. The human intestinal microbiota has extensive hydrolytic activities and breaks down many complex polyphenols into smaller phenolic acids, which can be absorbed across the intestinal mucosa. These polyphenol catabolites are only then available to exert their biological activities systemically within the host. However, to date, with the exception of a few compounds such as the phytoestrogens equol, enterolactone, and enterodiols, very little is known about the biological activities of phenolic acids derived from microbiota polyphenol metabolism.^{2,3} Table 1 presents some examples of parent plant polyphenols, microbial catabolites, and their potential biological effect when known. Given the diversity of plant polyphenols, their possible microbial catabolites, and the fact that they occur as mixtures in whole plant foods or plant extracts, only a small number of well-studied polyphenols are presented. Biological activities have not been described for most of the small

phenolic acid catabolites, but common activities recognized so far include antibacterial activities especially against Gram-negative species, like the Enterobacteriaceae, anti-inflammatory activities, anti-AGE formation, stimulation of xenobiotic degrading enzymes and detoxification processes, and phytoestrogenic activities.

Recently, Miene et al.²¹ demonstrated that microbial metabolites of quercetin and chlorogenic acid/caffeic acid, 3,4-dihydroxyphenylacetic acid (ES), and 3-(3,4-dihydroxyphenyl)propionic acid (PS), respectively, could significantly up-regulate GSTT2 expression and decrease COX-2 expression, a modulation seen as protective against colon cancer, and at the same time reduce DNA damage using an intestinal cancer cell line. González-Sarrías et al.²² showed that ellagic acid (EA) and its colonic metabolites, urolithin-A (3,8-dihydroxy-6H-dibenzo[*b,d*]pyran-6-one, Uro-A) and urolithin-B (3-hydroxy-6H-dibenzo[*b,d*]pyran-6-one, Uro-B), modulate the expression and activity of CYP1A1 and UGT1A10 and inhibit several sulfotransferases in colon cancer cell lines (Caco-2). These phase I and phase II detoxifying enzymes are important components of how our bodies deal with toxic and xenobiotic compounds, and increased expression is associated with a reduced risk of colon cancer in laboratory animals. However, these effects appeared to be critically affected by food matrix in the rat colon. Urolithins, from pomegranate, had, however, already been shown to reduce the growth of cancer cells in an animal model of prostate cancer.²³

The 3,4-dihydroxyphenylpropionic acid (3,4-DHPPA), 3-hydroxyphenylpropionic acid, and 3,4-dihydroxyphenylacetic acid (3,4-DHPAA), derived from colonic catabolism of proanthocyanidins, have been shown to reduce the inflammatory response of human peripheral blood mononuclear cells stimulated with lipopolysaccharide (LPS), an inflammatory cell wall component from Gram-negative bacteria such as the Enterobacteriaceae. Secretion of IL-6, IL-1, and TNF- α was reduced, suggesting that microbial metabolites may be involved in dampening the inflammatory response to bacterial antigens, which may have implications for chronic inflammatory or autoimmune diseases such as IBD.²⁴ Upon screening 18 microbial catabolites of polyphenols for their anti-inflammatory potential in vitro, Larrosa et al.²⁵ found that hydrocaffeic (HCAF), dihydroxyphenylacetic (dOHPA), and hydroferulic acid (HFER) reduced prostaglandin E(2) production by at least 50% in CCD-18 colon fibroblast cells stimulated with IL-1 β . The same compounds were also shown to reduce inflammation in rodents, and HCAF was also shown to reduce intestinal inflammation in the DSS-mouse model of ulcerative colitis with reduced mucosal expression of cytokines IL-1 β , IL-8, and TNF- α , reduced malonyldialdehyde (MDA) levels, and decreased oxidative DNA damage (measured as 8-oxo-2'-deoxyguanosine levels). Radnai et al.²⁶ also showed that ferulaldehyde, a microbial catabolite of curcumin, has anti-inflammatory properties in vivo in an animal model of LPS-induced septic shock. Intraperitoneal injection of ferulaldehyde (6 mg/kg) reduced markers of inflammation and prolonged the lifespan of LPS-treated animals, indicating that microbial catabolites might, at least in part, account for the observed anti-inflammatory activity of certain herbal medicines and functional foods.

Microbial catabolites of plant polyphenols may also affect disease risk in metabolic syndrome, a cluster of biomarkers associated with obesity and increased risk of cardiovascular disease and diabetes. Verzelloni et al.²⁷ demonstrated that urolithins and pyrogallol, microbial ellagitannin-derived catab-

olites, are highly antiglycative compared to parent polyphenolic compounds in an in vitro model of protein glycation. Protein glycation plays an important pathological role in diabetes and the pathologies associated with diabetes including blindness. They also found that chlorogenic acid-microbially derived catabolites, dihydrocaffeic acid, dihydroferulic acid, and feruloylglycine, were most effective at protecting cultured neural cells in vitro, indicating that colonic catabolites of dietary polyphenols may play an important role in the improved cognitive function and protection from neuronal degeneration observed in animals fed polyphenol-rich foods such as certain berries.

Plant polyphenols have also been shown to have a direct effect on carbohydrate fermentation by the human gut microbiota in vitro. Using anaerobic fecal batch cultures, Bazzocca et al.²⁸ found that apple proanthocyanidins inhibited both metabolic degradation of short proanthocyanidins and SCFA production. On its face, such an observation may suggest that polyphenols might work against production of beneficial microbial metabolites within the colon. As discussed above, in individuals on a Western style, low-fiber diet, the proximal colon is the major site of saccharolytic fermentation, with potentially damaging proteolytic fermentation increasing distally as carbohydrate substrate becomes limiting. Retardation of carbohydrate fermentation in the proximal colon may extend SCFA production to the distal colon, thereby reducing the harmful effects of amino acid catabolites. However, this remains to be investigated either in vivo or using complex multistage continuous culture models of the colonic microbiota. Similarly, polyphenols have also been shown to directly affect the relative abundance of different bacteria within the gut microbiota with tea polyphenols and their derivatives reducing numbers of potential pathogens including *Clostridium perfringens* and *C. difficile* and certain Gram-negative *Bacteroides* spp., with less inhibition toward beneficial clostridia, bifidobacteria, and lactobacilli.²⁹

Human data on the impact of polyphenols on the gut microbiota are scarce. One recent work from the University of Reading has shown that a high cocoa-flavanol (494 mg/day) drink can mediate a prebiotic type modulation of the gut microbiota in a randomized intervention study compared to a low cocoa-flavanol (23 mg/day) drink. The high-flavanol drink resulted in significant increases in fecal bifidobacteria and lactobacilli and a reduction in clostridial counts and a concomitant reduction in plasma triglycerides and C-reactive protein, both important markers of metabolic disease.³⁰ These results were mirrored by previous in vitro studies using fecal batch cultures by the same group.³¹

■ WHOLE PLANT FOODS AND THE GUT MICROBIOTA

Although much work has been conducted on the effect of isolated fibers on the composition and activity of the human gut microbiota, few studies have examined the impact of whole plant foods on gut microbial species composition and relative abundance. Recently, whole grain breakfast cereals have been shown to mediate a prebiotic modulation of the gut microbiota, giving significant increases in fecal bifidobacteria and/or lactobacilli without changing the relative abundance of other dominant members of the gut microbiota.^{32,33} Costabile et al.³² also showed that ingestion of either whole grain wheat or wheat bran breakfast cereal increased plasma and urine concentrations of ferulic acid, a polyphenol commonly complexed with dietary

fiber in whole grain cereals. In a small scale ($n = 8$) observational study, two apples per day for 2 weeks was also shown to increase numbers of fecal bifidobacteria, lactobacilli, and streptococci/enterococci while reducing numbers of enterobacteria and lecithinase-positive clostridia, including *C. perfringens* and *Pseudomonas* species.³⁴ However, the study did not use culture-independent microbiology techniques and suffered from the lack of a control group. Gill et al.³⁵ did not observe any discernible change in fecal bacterial populations in another small human feeding study ($n = 10$) with raspberry puree (200 g/day for 4 days) due to high intersubject variation in fecal bacteria but did report that microbial raspberry polyphenol metabolite profiles varied greatly between individuals, indicating that species makeup of the gut microbiota may affect catabolite profiles released by bacteria in the colon. In a recent large-scale dietary intervention, the FLAVURS group investigated the relative impact of increased fruit and vegetable intake of differing flavonoid content on markers of cardiovascular disease risk.³⁶ One hundred and fifty-four subjects at risk of cardiovascular disease were divided into three parallel groups consuming either their habitual diet, a high-flavonoid fruit and vegetable diet, or a low-flavonoid fruit and vegetable diet for 18 weeks. Subjects on the fruit and vegetable diets consumed two 80 g portions per day for 6 weeks, increasing to four portions and then six portions for the following two 6 week periods. The dietary objectives were broadly met by the subjects with the inclusion of complex and processed foods containing flavonoids, and significant increases in urinary total flavonoids were observed for the high-flavonoid group, whereas both fruit and vegetable diets gave dose-proportional increases in plasma vitamin C and other phytochemicals. Nonstarch polysaccharides, a measure of dietary fiber, also increased in both fruit and vegetable treatment groups as reported by diet diaries. Culture-independent analysis of fecal bacteria revealed that in the first cohort ($n = 59$), dietary intervention with either flavonoid-rich or flavonoid-poor fruits and vegetables significantly increased groups of commensal bacteria important for human health including *Bifidobacterium*, *Atopobium*, *Ruminococcus*, *Roseburia*, *Eubacterium*, and *Faecalibacterium prausnitzii*, whereas the flavonoid-poor diet also increased lactobacilli compared to the control diet. No changes in gut microbiota profile occurred in the group following the control diet and fecal SCFA did not change. Currently, at Fondazione Edmund Mach in Italy, we are conducting a full metabolomics profile of biofluids and gut metagenomic analysis of samples collected from these subjects to assess the impact of increased fruit and vegetable intake on the gut microbiota and how this may affect systemic metabolism via up-regulation of the metabolite flux from the colon.

DISCUSSION

There is growing evidence from animal and human studies that the two main classes of biologically active molecules in whole plant foods, dietary fibers and polyphenols, can have dramatic effects on the human gut microbiota. These compounds appear to modulate both species composition within the gut microbiota and the profile of metabolites absorbed from the colon. However, to date, few studies in humans have focused on the possible biological activities of the small phenolic catabolites produced by gut microbiota transformation of plant polyphenols. Similarly, few studies have considered the possible synergistic activity of fiber and polyphenols present in whole

plant foods. From the limited number of intervention studies with whole grain cereals that also measured gut microbiota effects, it appears that increasing consumption of whole plant foods might significantly up-regulate groups of commensal bacteria thought to be associated with human health. There is, however, a dearth of randomized, controlled, human intervention studies with whole plant foods that include measures of the gut microbiota or their metabolic output. This current reality is limiting full appreciation of how the closely coevolved relationship between whole plant foods and our gut microbiota regulates human health.

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Notes

The authors declare no competing financial interest.

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